Electronic Supplementary Information

DNA pseudoknots-functionalized sensing platform for chemoselective analysis of mercury ions

Libing Fu, Junyang Zhuang, Dianping Tang*, Xiaohua Que, Wenqiang Lai and Guonan Chen*

Key Laboratory of Analysis and Detection of Food Safety (Ministry of Education & Fujian Province), Department of Chemistry, Fuzhou University, Fuzhou 350108, P.R. China.

Fax: +86 591 22866135; Tel: +86 591 22866125;

E-mails: dianping.tang@fzu.edu.cn (D. Tang), gnchen@fzu.edu.cn (G. Chen)

EXPERIMENTAL SECTION

Reagents. 6-mercaptohexanol (MCH) and $NaClO_4$ were purchased from Dingguo Biotechnol. Co. Ltd (Beijing, China). The DNA pseudoknots labeled with ferrocene (Fc) and –SH group, and the auxiliary DNA strands were purchased from Sangon Biotechnol. Co. Ltd. (Shanghai, China). The sequences are as follows:

(1) DNA pseudoknot:

5′-HS-$(CH_2)_6$-GGTCGTGGTAGAT*CGTCGGC*CAGCCACGACGAAAAAAAAAAAAAA*GCCG ACG*-$(CH_2)_6$-Fc-3′ (*Note*: In the pseudoknots, the underlined bases and the italicized bases are complementary, respectively)

(2) Auxiliary DNA strand: 5′-TCGTTCTTCCTCGTC-3′

Both oligonucleotides stock solutions (5 μM) were prepared in Tris–HCl solution (20 mM Tris–HCl, pH 7.4). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. Phosphate-buffered saline (PBS, 0.1 M, pH 7.4) was prepared by adding 12.2 g K_2HPO_4, 1.36 g KH_2PO_4, and 8.5 g NaCl into 1000 mL deionized water.

Preparation of Electrochemical Sensor. A gold electrode (3.0 mm in diameter) was polished repeatedly with 1.0, 0.3 μm alumina slurry, followed by successive sonication in bi-distilled water and ethanol for 5 min and dried in air. Prior to the experiment, the gold electrodes were cleaned with hot piranha solution (a 3:1 mixture of H_2SO_4 and H_2O_2. *Cautions*!) for 10 min, and then continuously scanned within the potential range of 0 to 1.5 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the clean gold electrode was established. After washing with distilled water, 10 μL of DNA pseudoknot solution (0.5 μM) was dropped onto the surface of the freshly cleaned gold electrode that was held upside down, and kept in a water-saturated atmosphere for 8 h at 37 °C. During this process, the pseudoknots were assembled on the gold electrode through –Au-S- binding. Excessive pseudoknots were removed by washing with pH 7.4 PBS. Afterwards, the pseudoknot-functionalized gold electrode was suspended into 1.0 mM 6-mercaptohexanol (MCH) solution for 2 h at room temperature in order to make the pseudoknots were aligned and nearly perpendicular to the surface of gold electrode. Finally, the

as-prepared sensor was stored at 4 °C for further usage.

Electrochemical Measurement. The analytical procedure for detection of Hg^{2+} is schematically depicted in Scheme 1. Electrochemical experiments were conducted on CHI 620D electrochemical workstation (Shanghai CH Instruments Inc., China) using a conventional three-electrode system with a conventional three-electrode system with a modified gold electrode as working electrode, a platinum foil as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. Initially, the pseudoknot-modified gold electrode was immersed into the incubation solution containing 5 μM auxiliary DNA oligonucleotide and different-concentration Hg^{2+} samples/standards, and then incubated for 50 min at 37 °C. After washing with pH 7.4 PBS, the resulting electrode was placed into 0.1 M PBS (pH 7.4) containing 0.1 M $NaClO_4$. Meanwhile, square wave voltammetry (SWV) from 600 mV to 0 mV (*vs.* SCE) (Amplitude: 25 mV; Frequency: 15 Hz; Increase *E*: 4 mV) was collected and registered as the sensor signals. Analyses are always made in triplicate. The peak current observed at 0.25 V was used to estimate the analytical characteristics of the sensor. The electrochemical signal provided in this work was recorded by drawing a tangent between both sides of current peak.

Analysis of Surface Coverage of DNA Pseudoknots. The surface coverage of DNA pseudoknots on the gold electrode can be calculated from the number of cationic redox marker measured at the electrode surface. The saturated amount of charge-compensation redox marker in the DNA monolayer can be determined by using chronocoulometry in 10 mM Tris buffer, pH 7.4 (E-BFR), which is directly proportional to the number of phosphate residues and thereby the surface density of DNA. The surface excess of hexamine ruthenium (III) chloride (RuHex) $[Ru(NH_3)_6]^{3+}$) at a DNA-modified electrode is determined from the difference in intercepts for the response in the absence and presence of redox marker. Surface densities of single-stranded DNA were precisely varied in the range of $(1-10) \times 10^{12}$ molecules cm^{-2}, as determined by the electrochemical method, using mixed monolayers.

Cations provide charge compensation for the anionic phosphate groups in DNA. When an electrode modified with DNA is placed in a low ionic strength electrolyte containing a multivalent redox cation. The amount of cationic redox marker can be determined using chronocoulometry, a

current integration technique, under equilibrium conditions. The integrated current, or charge Q, as a function of time (t) in a chronocoulometric experiment is given by the integrated Cottrell expression:

$$Q = (2nFAD_0^{1/2}C_0^*/\prod^{1/2})t^{1/2} + Q_{dl} + nFA\,\Gamma_0 \quad (1)$$

Where n is the number of electrons per molecule for reduction, C_o* the bulk concentration (mol cm^{-2}), D_0 the diffusion coefficient (cm^2 s^{-1}), F the Faraday constant (C/equiv), Q_{dl} the capacitive charge (C), A the electrode area (cm^2), and $nFA\Gamma_0$ the charge from the reduction of Γ_0 (mol cm^{-2}) of adsorbed redox marker. The term Γ_0 designates the surface excess and represents the amount of redox marker confined near the electrode surface. The chronocoulometric intercept at t = 0 is then the sum of the double-layer charging and the surface excess terms. The surface excess is determined from the difference in chronocoulometric intercepts for the identical potential step experiment in the presence and absence of redox marker.

The saturated surface excess of redox marker is converted to DNA probe surface density with the relationship:

$$\Gamma_{DNA} = \Gamma_0(z/m)(N_A) \quad (2)$$

Where Γ_{DNA} is the probe surface density in molecules cm^{-2}, z is the charge of the redox molecule, m is the number of bases in the probe DNA, and N_A is Avogadro's number.

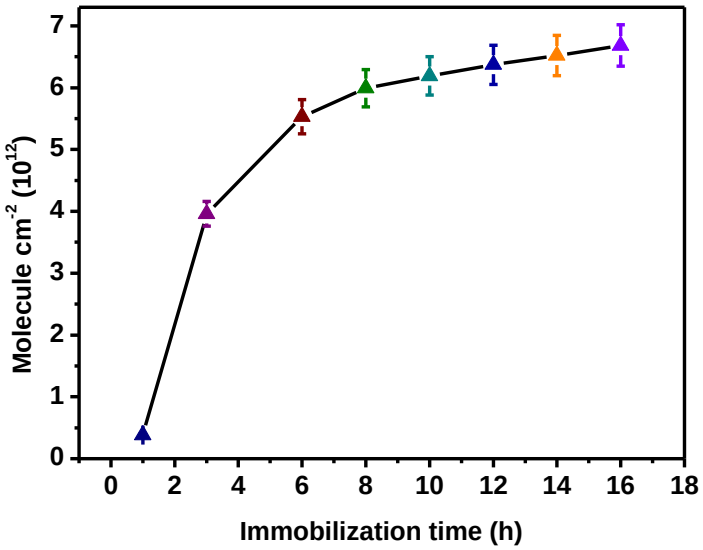

Fig. S1 The binding density of DNA pseudoknots (0.5 μM) on the gold electrode at various incubation times.

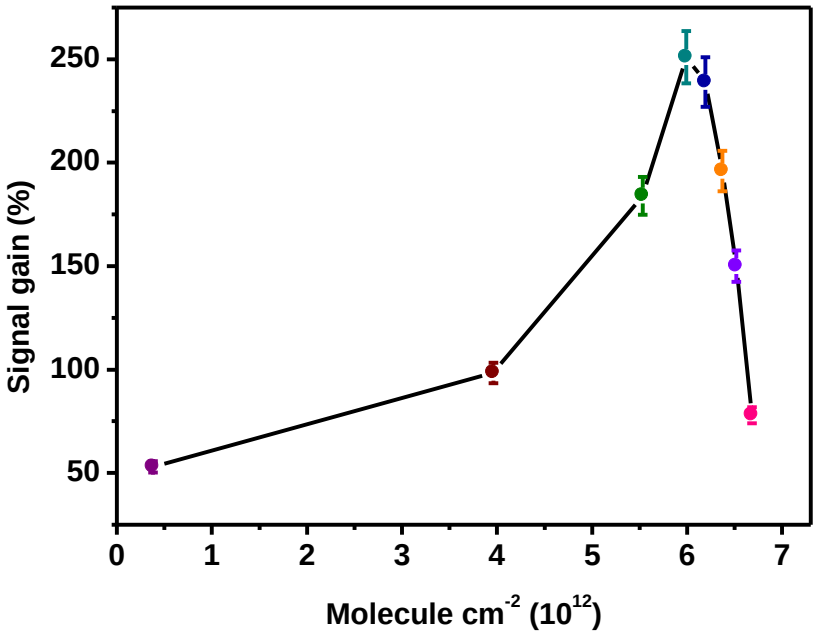

Fig. S2 The influence of differently binding densities on the electrochemical signal of the sensor (1.0 μM Hg^{2+} used in this case).

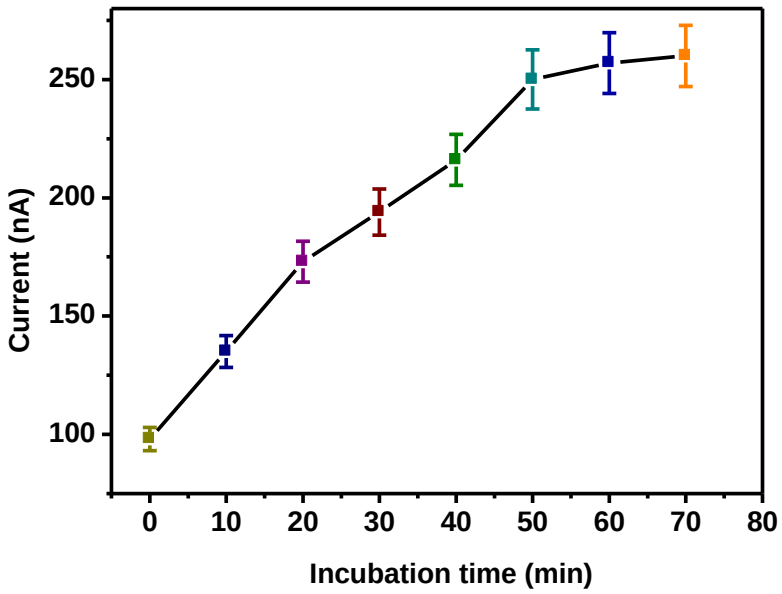

Fig. S3 The influence of the hybridization time between the sensor and the incubation solution (i.e. auxiliary strand + Hg^{2+}) on the electrochemical signal of the sensor (1.0 μM Hg^{2+} used in this case).

Table S1

Comparison of analytical properties of the pseudoknot-based Hg^{2+} electrochemical sensor with other Hg^{2+} detection methods.

Method	Linear range	Detection limit	Ref.
Photoelectrochemical DNA sensor	0.1 nM-10 nM	20 pM	[S1]
Ratiometric fluorescence sensor	-	200 nM	[S2]
Quantum dots-based fluorescence assay	8.0 nM-2.0 μM	2.0 nM	[S3]
Electrochemiluminescence nanoprobe	0.05 nM-100 nM	50 pM	[S4]
Colorimetric sensor	-	10 nM	[S5]
Fluorescence sensing strategy	-	0.2 nM	[S6]
Fluorescence sensing strategy	-	0.23 nM	[S7]
Test strip-based colorimetric sensor	3.0 nM-100 nM	3.0 nM	[S8]
Electrochemical sensor	0.1 nM-10 μM	52 pM	[S9]
Surface-enhanced Raman scattering	30 nM-150 nM	30 nM	[S10]
Pseudoknot-based Hg^{2+} electrochemical sensor	0.01 nM-10 μM	6.0 pM	This work

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S3 Y. Yu, R. Zhang, K. Zhang and S. Sun, *J. Nanosci. Nanotechnol.*, 2012, **12**, 2783.

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Table S2

Comparison of the assay results for real water samples using the developed Hg^{2+} sensor and the referenced ICP-MS.

Sample no.[a]	Method; Concentration (mean ± SD, n = 3, nM)[b]		t_{exp}
	Hg^{2+} sensor	ICP-MS	
1	0.08 ± 0.02	0.09 ± 0.01	0.77
2	50.6 ± 2.4	46.8 ± 2.7	1.82
3	376.4 ± 1.2	378.1 ± 1.1	1.81
4	14.6 ± 0.8	13.4 ± 0.5	2.21
5	28.5 ± 1.3	30.7 ± 1.1	2.24
6	24.6 ± 1.1	26.1 ± 0.9	1.83

[a] Samples 1-3 were the spiked river water, while samples 4-6 were the contaminated sewage.

[b] The regression equation (linear) for these data is as follows: $y = 1.0047x - 0.3209$ ($R^2 = 0.9998$) (x-axis: by the pseudoknot-based electrochemical sensor y-axis: by the referenced ICP-MS).